

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions of the claims and listing of the claims in the application:

1. **(Currently Amended)** An isolated α -keto acid reductase having the following physicochemical properties:

(i) function:

reduces α -keto acid to (R)- α -hydroxy acid using reduced β -nicotinamide adenine dinucleotide as the coenzyme; and

(ii) substrate specificity:

(a) utilizes reduced β -nicotinamide adenine dinucleotide as the coenzyme in the reduction reaction of (i);

(b) reduces 2-chlorophenyl glyoxylic acid to (R)-2-chloromandelic acid; and

(c) reduces 2-chlorophenyl glyoxylic acid but the dehydrogenase activity of an enzyme against either of and dehydrogenates the two optical isomers of 2-chloromandelic acid is no more than 20% taking the relative activity of the enzyme to reduce compared to the dehydrogenation of 2-chlorophenyl glyoxylic acid,

wherein said α -keto acid reductase is encoded by a polynucleotide selected from the group consisting of:

(1) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:1;

(2) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2;

(3) a polynucleotide encoding an amino acid sequence comprising an amino acid sequence at least 95% homologous to the amino acid sequence of SEQ ID NO:2.

2. **(Previously Presented)** The isolated α -keto acid reductase of claim 1, further having the following physicochemical properties:

(iii) optimum pH:

pH 5.0 to 5.5;

(iv) optimum temperature:

45 to 55°C; and

(v) molecular weight of

about 35,000 Daltons and about 63,000 Daltons, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and gel filtration, respectively.

3. **(Previously Presented)** The isolated α -keto acid reductase of claim 1, which is produced by a microorganism belonging to the genus *Leuconostoc*.
4. **(Previously Presented)** The isolated α -keto acid reductase of claim 3, wherein the microorganism belonging to the genus *Leuconostoc* is *Leuconostoc mesenteroides*.
5. **(Previously Presented)** The isolated α -keto acid reductase of claim 4, wherein the microorganism belonging to *Leuconostoc mesenteroides* is *Leuconostoc mesenteroides* subsp. *dextranicum*.
6. **(Withdrawn)** A polynucleotide encoding a protein, wherein said protein is an enzyme that catalyzes the reduction of α -keto acids, and wherein said polynucleotide is selected from the group consisting of:
- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1;
 - (b) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2;
 - (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, wherein one or more amino acids have been substituted, deleted, inserted, and/or added;
 - (d) a polynucleotide hybridizing under stringent conditions to a DNA comprising the nucleotide sequence of SEQ ID NO: 1; and
 - (e) a polynucleotide encoding an amino acid sequence which exhibits 50% or higher homology to the amino acid sequence of SEQ ID NO: 2.
7. **(Previously Presented)** An isolated protein, wherein said protein is an enzyme that catalyzes the reduction of α -keto acids, and wherein said protein is encoded by a polynucleotide selected from the group consisting of:
- (1) a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1;

(2) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:2.

8. **(Withdrawn)** A recombinant vector wherein the polynucleotide of claim 6 has been inserted.

9. **(Withdrawn)** The recombinant vector of claim 8, wherein a polynucleotide encoding a dehydrogenase catalyzing an oxidation-reduction reaction using β -nicotinamide adenine dinucleotide as the coenzyme has been further inserted.

10. **(Withdrawn)** The vector of claim 9, wherein the dehydrogenase is a formate dehydrogenase.

11. **(Withdrawn)** The vector of claim 10, wherein the formate dehydrogenase is derived from *Mycobacterium vaccae*.

12. **(Withdrawn)** The vector of claim 9, wherein the dehydrogenase is a glucose dehydrogenase.

13. **(Withdrawn)** The recombinant vector of claim 12, wherein the glucose dehydrogenase is derived from *Bacillus subtilis*.

14. **(Withdrawn)** A transformant comprising any one of the polynucleotides of claim 6 in an expressible manner.

15. **(Withdrawn)** A method for producing the protein of claim 7, wherein said method comprises the steps of culturing a transformant comprising any one of the polynucleotides selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1;

(b) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2;

(c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, wherein one or more amino acids have been substituted, deleted, inserted, and/or added;

(d) a polynucleotide hybridizing under stringent conditions to a DNA comprising the nucleotide sequence of SEQ ID NO: 1; and

(e) a polynucleotide encoding an amino acid sequence which exhibits 50% or higher homology to the amino acid sequence of SEQ ID NO: 2,

and collecting the expressed product.

16. **(Withdrawn)** A method for producing the enzyme of claim 1, wherein said method comprises the step of culturing a microorganism belonging to the genus *Leuconostoc*.

17. **(Withdrawn)** The method of claim 16, wherein the microorganism belonging to the genus *Leuconostoc* is *Leuconostoc mesenteroides*.

18. **(Withdrawn)** The method of claim 17, wherein the microorganism belonging to *Leuconostoc mesenteroides* is *Leuconostoc mesenteroides* subsp. *dextranicum*.

19. **(Withdrawn)** A method for producing an optically active α -hydroxy acid, wherein said method comprises the following sequential steps:

(i) reacting

(a) the α -keto acid reductase of claim 1;

(b) a protein encoded by a polynucleotide selected from the group consisting of:

(1) a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1;

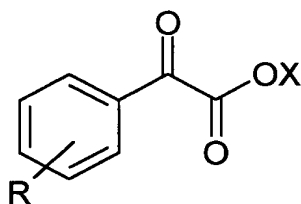
(2) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2;

(3) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, wherein one or more amino acids have been substituted, deleted, inserted, and/or added;

(4) a polynucleotide hybridizing under stringent conditions to a DNA comprising the nucleotide sequence of SEQ ID NO: 1; and

- (5) a polynucleotide encoding an amino acid sequence which exhibits 50% or higher homology to the amino acid sequence of SEQ ID NO: 2;
- (c) a microorganism producing said α -keto reductase or said protein; or
- (d) a processed product of the microorganism
- with an α -keto acid; and
- (ii) collecting the optically active α -hydroxy acid produced in step (i).

20. **(Withdrawn)** The method of claim 19, wherein the α -keto acid is a phenylglyoxylic acid derivative of formula (I):



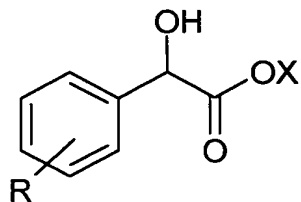
formula (I)

wherein:

X is a hydrogen atom, an alkaline metal, or a alkaline earth metal; and

R indicates one or more substituents at the ortho, meta, or para positions selected from the group consisting of a halogen atom, a hydroxyl group, a C₁₋₃ alkyl group, a C₁₋₃ alkoxy group, a C₁₋₃ thioalkyl group, an amino group, a nitro group, a mercapto group, a phenyl group, and a phenoxy group,

and wherein said method comprises the step of collecting the optically produced active mandelic acid derivative of formula (II):



formula (II)

wherein X and R are as defined in Formula (I).

21. **(Withdrawn)** The method of claim 20, wherein the ortho position of the phenylglyoxylic acid derivative is substituted.
22. **(Withdrawn)** The method of claim 21, wherein the ortho position of the phenylglyoxylic acid derivative is substituted with a halogen atom.
23. **(Withdrawn)** The method of claim 20, wherein the meta position of the phenylglyoxylic acid derivative is substituted.
24. **(Withdrawn)** The method of claim 23, wherein the meta position of the phenylglyoxylic acid derivative is substituted with a halogen atom.
25. **(Withdrawn)** The method of claim 19, wherein the α -keto acid is 2-chlorophenyl glyoxylic acid and the optically active α -hydroxy acid is (R)-2-chloromandelic acid.
26. **(Withdrawn)** The method of claim 19, wherein the microorganism is a transformant any one of the polynucleotides selected from the group consisting of
- (a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO: 1;
 - (b) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2;
 - (c) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO: 2, wherein one or more amino acids have been substituted, deleted, inserted, and/or added;
 - (d) a polynucleotide hybridizing under stringent conditions to a DNA comprising the nucleotide sequence of SEQ ID NO: 1; and
 - (e) a polynucleotide encoding an amino acid sequence which exhibits 50% or higher homology to the amino acid sequence of SEQ ID NO: 2.

27. **(Withdrawn)** The method of claim 19, wherein said method further comprises the step of converting oxidized β -nicotinamide adenine dinucleotide to reduced β -nicotinamide adenine dinucleotide.

28. **(Withdrawn)** The method of claim 27, wherein the oxidized β -nicotinamide adenine dinucleotide is converted to reduced β -nicotinamide adenine dinucleotide by the function of an enzyme that catalyzes dehydrogenation using oxidized β -nicotinamide adenine dinucleotide as the coenzyme.

29. **(Withdrawn)** The method of claim 28, wherein the enzyme that catalyzes dehydrogenation using oxidized β -nicotinamide adenine dinucleotide as the coenzyme is formate dehydrogenase and/or glucose dehydrogenase.